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USPT

US-PAT-NO: 5270030
DOCUMENT-IDENTIFIER: US 5270030 A
TITLE: Fibrin binding domain polypeptide and method of producing
DATE-ISSUED: December 14, 1993
INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Vogel; Tikva	Rehovot	N/A	N/A	ILX
Levanon; Avigdor	Rehovot	N/A	N/A	ILX
Werber; Moshe M.	Tel Aviv	N/A	N/A	ILX
Guy; Rachel	Rehovot	N/A	N/A	ILX
Panet; Amos	Jerusalem	N/A	N/A	ILX

ASSIGNEE INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY	TYPE CODE
Bio-Technology General Corp.	New York	NY	N/A	N/A	02

APPL-NO: 7/ 526397
DATE FILED: May 21, 1990
PARENT-CASE:
This application is a continuation-in-part of U.S. Ser. No. 345,952, filed
Apr. 28, 1989, now abandoned, which is a continuation-in-part of U.S. Ser.
No. 291,951, filed Dec. 29, 1988, now abandoned, the contents of both of which
are hereby incorporated by reference into this application.
FOREIGN-APPL-PRIORITY-DATA:
FOREIGN-PRIORITY:
FOREIGN-PRIORITY-APPL-NO: CA 2006929
FOREIGN-PRIORITY-APPL-DATE: December 29, 1989
INT-CL: [5] A61K099/00,C07K013/00 ,C12N015/74 ,C12P021/02
US-CL-ISSUED: 424/9,530/350 ,530/395 ,435/320.1 ,435/69.1 ,435/252.3
,435/252.33 ,435/240.1 ,424/1.1
US-CL-CURRENT: 424/1.69,424/9.341 ,424/9.4 ,435/252.3 ,435/252.33
,435/320.1
,435/69.1 ,530/350 ,530/395
FIELD-OF-SEARCH: 530/350;530/395 ;435/320.1 ;435/69.1 ;435/252.3
;435/252.33
;435/240.1 ;424/9 ;424/1.1 ;534/10 ;436/173 ;128/653.4

REF-CITED:

U.S. PATENT DOCUMENTS

PAT-NO	ISSUE-DATE	PATENTEE-NAME	US-CL
4315906	February 1982	Gelder	424/1
4455290	June 1984	Olexa et al.	424/1.1
4587122	May 1986	Kagitani et al.	424/101
4663146	May 1987	Morser et al.	424/1.1
4839464	June 1989	McCarthy et al.	530/326

FOREIGN PATENT DOCUMENTS

COUNTRY	FOREIGN-PAT-NO	PUBN-DATE	US-CL
EP	0207751	January 1987	530/350
JP	1261398	October 1989	
WO	WO89/00051	January 1989	

ART-UNIT: 182

PRIMARY-EXAMINER: Ossanna; Nina

ATTY-AGENT-FIRM: White; John P.

ABSTRACT:

This invention provides an imaging agent which comprises a polypeptide labeled with an imageable marker, such polypeptide having an amino acid sequence substantially present in the fibrin binding domain of naturally-occurring human fibronectin and being capable of binding to fibrin. The invention further provides a method wherein the imaging agent is used for imaging a fibrin-containing substance, i.e., a thrombus or atherosclerotic plaque. Further provided are plasmids for expression of polypeptides having an amino acid sequence substantially present in the fibrin binding domain of naturally-occurring human fibronectin and being capable of binding to fibrin, hosts containing these plasmids, methods of producing the polypeptides, methods of treatment using the polypeptides, and methods of recovering, refolding and reoxidizing the polypeptides. The invention also provides for purified polypeptides substantially free of other substances of human origin which have an amino acid sequence substantially present in the fibrin binding domain of naturally-occurring human fibronectin and which are capable of binding to fibrin.

Case 19 R's file

15 Claims, 62 Drawing figures

CLAIMS:

What is claimed is:

1. An imaging agent which comprises a polypeptide labeled with an imageable marker, wherein the polypeptide is a 12 kD polypeptide corresponding to an amino acid sequence present in the fibrin binding domain of naturally-occurring human fibronectin and having the amino acid sequence of amino acids 1-109 as shown in FIG. 1 and being capable of binding to fibrin.

2. A composition comprising an effective imaging amount of the imaging agent of claim 1 and a physiologically acceptable carrier.

3. An agent of claim 1, wherein the marker is a radioactive isotope, an element which is opaque to X-rays, or a paramagnetic ion.

4. An agent of claim 3, wherein the marker is a radioactive isotope.

5. An agent of claim 4, wherein the radioactive isotope is indium-111.

6. An agent of claim 4, wherein the radioactive isotope is technetium-99m.

7. An agent of claim 4, wherein the radioactive isotope is iodine-123, iodine-125, iodine-131, krypton-81m, xenon-133, or gallium-67.

8. A purified polypeptide substantially free of other substances of human origin wherein the polypeptide is a 12 kD polypeptide of amino acids 1-109 as

shown in FIG. 1 corresponding to an amino acid sequence present in the fibrin
binding domain of naturally-occurring human fibronectin and being capable of
binding to fibrin.

9. A plasmid for expression of the polypeptide of claim 8 comprising DNA
encoding the polypeptide and DNA encoding suitable regulatory elements
positioned relative to the DNA encoding the polypeptide so as to effect
expression of the polypeptide in a suitable host cell.

10. A plasmid according to claim 9 designated pFN 196-2 and deposited in
escherichia coli strain A4255 under ATCC Accession No. 63328.

11. A cell which comprises the plasmid of claim 9.

12. A bacterial cell according to claim 11.

13. An Escherichia coli cell according to claim 12.

14. An Escherichia coli cell according to claim 13, wherein the plasmid is
designated pFN 196-2 and wherein the cell is deposited under ATCC Acession No.
68328.

15. A method of producing a 12 kD polypeptide fragment corresponding to an
amino acid sequence present in the fibrin binding domain of naturally-occurring
human fibronectin which comprises culturing a cell according to claim 11 so
that the DNA directs expression of the polypeptide and the cell expressed the
polypeptide and recovering from the cell the polypeptide so expressed.

USPT

US-PAT-NO: 5455158
DOCUMENT-IDENTIFIER: US 5455158 A
TITLE: Fibrin binding domain polypeptides and uses and methods of producing
same
DATE-ISSUED: October 3, 1995
INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Vogel; Tikva	Rehovot	N/A	N/A	ILX
Levanon; Avigdor	Rehovot	N/A	N/A	ILX
Werber; Moshe M.	Tel Aviv	N/A	N/A	ILX
Guy; Rachel	Rehovot	N/A	N/A	ILX
Panet; Amos	Jerusalem	N/A	N/A	ILX

ASSIGNEE INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY	TYPE CODE
Bio-Technology General Corp.	Iselin	NJ	N/A	N/A	02

APPL-NO: 8/ 058241
DATE FILED: May 4, 1993
PARENT-CASE:
This application is a divisional of U.S. Ser. No. 07/526,397, filed May 21,
1990, now U.S. Pat. No. 5,270,030; which is a continuation-in-part of U.S.
Ser. No. 07/345,952, filed Apr. 28, 1989, now abandoned; which was a
continuation-in-part of U.S. Ser. No. 07/291,951, filed Dec. 29, 1988, now
abandoned.
FOREIGN-APPL-PRIORITY-DATA:
FOREIGN-PRIORITY:
FOREIGN-PRIORITY-APPL-NO: CA 2006929
FOREIGN-PRIORITY-APPL-DATE: December 29, 1989
INT-CL: [6] G01N033/53,A61K051/08 ,C12Q001/56
US-CL-ISSUED: 435/7.21,424/1.69 ,424/9.341 ,424/9.4 ,435/7.8 ,435/13 ,436/503
,436/504 ,436/69
US-CL-CURRENT: 435/7.21,424/1.69 ,424/9.341 ,424/9.4 ,435/13 ,435/7.8 ,436/503

,436/504 ,436/69
FIELD-OF-SEARCH: 435/7.21;435/13 ;435/7.8 ;436/503 ;436/504
;436/69 ;424/9
;424/1.1 ;424/1.69 ;530/380 ;530/381 ;530/350 ;514/2

REF-CITED:

U.S. PATENT DOCUMENTS

PAT-NO	ISSUE-DATE	PATENTEE-NAME	US-CL
4315906	February 1982	Gelder	424/9
5026537	June 1991	Daddiba et al.	424/1.1

FOREIGN PATENT DOCUMENTS

COUNTRY	FOREIGN-PAT-NO	PUBN-DATE	US-CL
EP	0207751	January 1987	

ART-UNIT: 182
PRIMARY-EXAMINER: Saunders; David
ASSISTANT-EXAMINER: Grun; James L.
ATTY-AGENT-FIRM: White; John P.

ABSTRACT:
This invention provides an imaging agent which comprises a polypeptide
labeled with an imageable marker, such polypeptide having an amino acid
sequence substantially present in the fibrin binding domain of
naturally-occurring human fibronectin and being capable of binding to fibrin.
The invention further provides a method wherein the imaging agent is used for
imaging a fibrin-containing substance, i.e., a thrombus or atherosclerotic
plaque. Further provided are plasmids for expression of polypeptides having an
amino acid sequence substantially present in the fibrin binding domain of
naturally-occurring human fibronectin and being capable of binding to fibrin,
hosts containing these plasmids, methods of producing the polypeptides, methods
of treatment using the polypeptides, and methods of recovering, refolding and
reoxidizing the polypeptides. The invention also provides for purified
polypeptides substantially free of other substances of human origin which have
an amino acid sequence substantially present in the fibrin binding domain of
naturally-occurring human fibronectin and which are capable of binding to
fibrin.

8 Claims, 98 Drawing figures

CLAIMS:

What is claimed is:

1. A method for imaging a fibrin-containing substance which comprises

contacting the fibrin-containing substance to be imaged with an imaging agent
under conditions such that the imaging agent binds to fibrin in the
fibrin-containing substance,

imaging bound imaging agent, and

thereby imaging the fibrin-containing substance,

wherein the imaging agent comprises a polypeptide labeled with an imageable
marker,

wherein the polypeptide is a 12 kD polypeptide corresponding to an amino acid
sequence present in the fibrin binding domain of naturally-occurring human
fibronectin and comprising the amino acid sequence of amino acids 1-109 as
shown in FIG. 1.

2. A method of claim 1, wherein the fibrin-containing substance is a thrombus.

3. A method of claim 1, wherein the fibrin-containing substance is
atherosclerotic plaque.

4. The method according to claim 1 wherein the fibrin-containing substance is
within blood vessels of a subject and wherein contacting is

performed by
administering the imaging agent contained in a suitable carrier to the subject
under conditions permitting the imaging agent to enter the blood vessels of the
subject.

5. A method of claim 4, wherein the fibrin-containing substance is a thrombus.

6. A method of claim 4, wherein the fibrin-containing substance is
atherosclerotic plaque.

7. A method of claim 1, wherein the marker is a radioactive isotope, an
element which is opaque to X-rays, or a paramagnetic ion.

8. A method of claim 1, wherein the imaging is carried out using a gamma
camera.

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L2: Entry 5 of 5 File: USPT Oct 3, 1995

US-PAT-NO: 5455158
DOCUMENT-IDENTIFIER: US 5455158 A

TITLE: Fibrin binding domain polypeptides and uses and methods of producing same

DATE-ISSUED: October 3, 1995

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Vogel; Tikva	Rehovot	N/A	N/A	ILX
Levanon; Avigdor	Rehovot	N/A	N/A	ILX
Werber; Moshe M.	Tel Aviv	N/A	N/A	ILX
Guy; Rachel	Rehovot	N/A	N/A	ILX
Panet; Amos	Jerusalem	N/A	N/A	ILX

US-CL-CURRENT: 435/7.21; 424/1.69, 424/9.341, 424/9.4, 435/13, 435/7.8, 436/503, 436/504, 436/69

CLAIMS:

What is claimed is:
1. A method for imaging a fibrin-containing substance which comprises
contacting the fibrin-containing substance to be imaged with an imaging agent under conditions such that the imaging agent binds to fibrin in the fibrin-containing substance,
imaging bound imaging agent, and
thereby imaging the fibrin-containing substance,
wherein the imaging agent comprises a polypeptide labeled with an imageable marker,
wherein the polypeptide is a 12 kD polypeptide corresponding to an amino acid sequence present in the fibrin binding domain of naturally-occurring human fibronectin and comprising the amino acid sequence of amino acids 1-109 as shown in FIG. 1.
2. A method of claim 1, wherein the fibrin-containing substance is a thrombus.
3. A method of claim 1, wherein the fibrin-containing substance is atherosclerotic plaque.
4. The method according to claim 1 wherein the fibrin-containing substance is within blood vessels of a subject and wherein contacting is performed by administering the imaging agent contained in a suitable carrier to the subject under conditions permitting the imaging agent to enter the blood vessels of the subject.
5. A method of claim 4, wherein the fibrin-containing substance is a thrombus.
6. A method of claim 4, wherein the fibrin-containing substance is atherosclerotic plaque.
7. A method of claim 1, wherein the marker is a radioactive isotope, an element which is opaque to X-rays, or a paramagnetic ion.
8. A method of claim 1, wherein the imaging is carried out using a gamma camera.

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L3: Entry 12 of 12 File: USPT Dec 14, 1993

US-PAT-NO: 5270030
DOCUMENT-IDENTIFIER: US 5270030 A

TITLE: Fibrin binding domain polypeptide and method of producing

DATE-ISSUED: December 14, 1993

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Vogel; Tikva	Rehovot	N/A	N/A	ILX
Levanon; Avigdor	Rehovot	N/A	N/A	ILX
Werber; Moshe M.	Tel Aviv	N/A	N/A	ILX
Guy; Rachel	Rehovot	N/A	N/A	ILX
Panet; Amos	Jerusalem	N/A	N/A	ILX

US-CL-CURRENT: 424/1.69; 424/9.341, 424/9.4, 435/252.3, 435/252.33, 435/320.1, 435/69.1, 530/350, 530/395

CLAIMS:

What is claimed is:
1. An imaging agent which comprises a polypeptide labeled with an imageable marker, wherein the polypeptide is a 12 kD polypeptide corresponding to an amino acid sequence present in the fibrin binding domain of naturally-occurring human fibronectin and having the amino acid sequence of amino acids 1-109 as shown in FIG. 1 and being capable of binding to fibrin.
2. A composition comprising an effective imaging amount of the imaging agent of claim 1 and a physiologically acceptable carrier.
3. An agent of claim 1, wherein the marker is a radioactive isotope, an element which is opaque to X-rays, or a paramagnetic ion.
4. An agent of claim 3, wherein the marker is a radioactive isotope.
5. An agent of claim 4, wherein the radioactive isotope is indium-111.
6. An agent of claim 4, wherein the radioactive isotope is technetium-99m.
7. An agent of claim 4, wherein the radioactive isotope is iodine-123, iodine-125, iodine-131, krypton-81m, xenon-133, or gallium-67.
8. A purified polypeptide substantially free of other substances of human origin wherein the polypeptide is a 12 kD polypeptide of amino acids 1-109 as shown in FIG. 1 corresponding to an amino acid sequence present in the fibrin binding domain of naturally-occurring human fibronectin and being capable of binding to fibrin.
9. A plasmid for expression of the polypeptide of claim 8 comprising DNA encoding the polypeptide and DNA encoding suitable regulatory elements positioned relative to the DNA encoding the polypeptide so as to effect expression of the polypeptide in a suitable host cell.
10. A plasmid according to claim 9 designated pFN 196-2 and deposited in escherichia coli strain A4255 under ATCC Accession No. 63328.
11. A cell which comprises the plasmid of claim 9.
12. A bacterial cell according to claim 11.
13. An Escherichia coli cell according to claim 12.
14. An Escherichia coli cell according to claim 13, wherein the plasmid is designated pFN 196-2 and wherein the cell is deposited under ATCC Acession No. 68328.
15. A method of producing a 12 kD polypeptide fragment corresponding to an amino acid sequence present in the fibrin binding domain of naturally-occurring human fibronectin which comprises culturing a cell according to claim 11 so that the DNA directs expression of the polypeptide and the cell expressed the polypeptide and recovering from the cell the polypeptide so expressed.